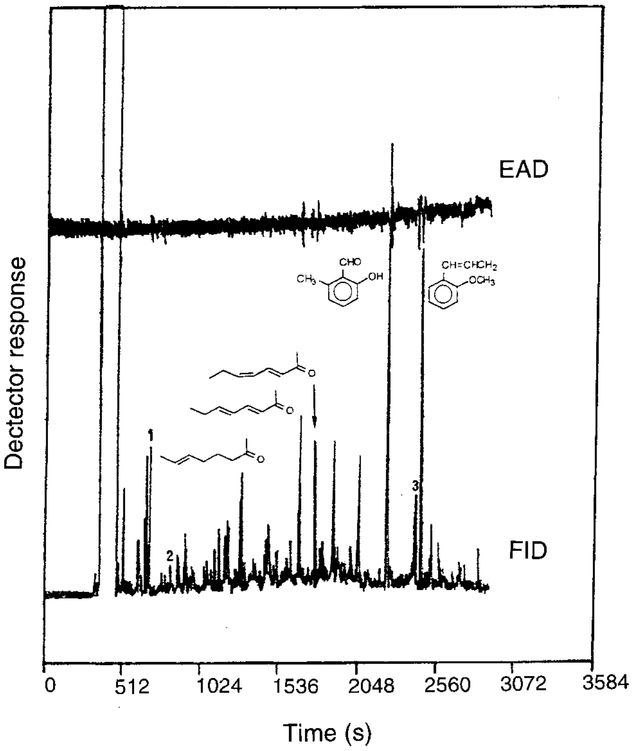

Figure 1. Representative reconstructed computer GC-EAD recordings of adult female *S gregaria* antennae, responding strongly to (*Z*)-6-octen-2-one, (*E,E*)-3,5-octadien-2-one, (*E,Z*)-3,5-octadien-2-one, 2-hydroxy-6-methylbenzaldehyde, 2-methoxy-1-propenyl-benzene and the unidentified compounds **1–3** in the crude volatile extract of three-month-old contaminated sand. Only the three unsaturated ketones evoke antennal responses in the volatile emissions of freshly contaminated sand.

octadien-2-one and its geometric isomer (*E,Z*)-3,5-octadien-2-one. Unlike acetophenone and veratrole, these compounds were traced to volatile emissions from the eggs. Analysis of volatiles from the sand stored for three months showed relatively lower levels of the three unsaturated ketones identified previously, compared to those present in freshly contaminated sand, which may account for the drop in activity of this sand. In addition, 14 other compounds which evoked antennal responses from adult females of *S gregaria* were detected. Of these, 2-hydroxy-6-methylbenzaldehyde, 2-methoxy-1-propenyl-benzene and three unidentified components (**1,2,3** Fig 1) evoked the strongest responses. Only trace amounts of the three unsaturated ketones were detected in the volatile emissions from the six-month-old sand which correlates with lack of activity of the sand. The results suggest that the aromatic compounds detected in EAG recordings in the volatiles of aged contaminated sand play no role in oviposition-site selection by gregarious females of *S gregaria*.

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Chemical interactions with the herbicide propanil on propanil-resistant barnyardgrass

Robert E Hoagland,[1]* Jason K Norsworthy[2] and Ronald E Talbert[2]
[1] *USDA-ARS, Southern Weed Science Research Unit, Stoneville, MS 38776, USA*
[2] *Agronomy Dept, University of Arkansas, Fayetteville, AR 72704, USA*

Abstract: We are examining the interaction of compounds with the herbicide propanil to find synergistic or additive actions that can increase efficacy against propanil-resistant barnyardgrass [*Echinochloa crus-galli*] (R-BYG) without substantial injury to rice. Field tests (herbicidal injury) and laboratory tests (chlorophyll quantification in excised leaves; measurement of chlorophyll fluorescence to determine PSII inhibition) have been conducted on R-BYG and rice tissue exposed to various rates of propanil and additive. Important synergistic interactions on R-BYG in laboratory and field tests were found with propanil plus either the herbicides anilophos or piperophos, or the insecticide carbaryl. In laboratory tests, the insecticide methiocarb and PPG-124 (*p*-chlorophenyl *N*-methylcarbamate) were highly effective synergists with propanil on R-BYG. Other important interactions occurred with certain concentrations/application rates when propanil was combined with the herbicides quinclorac, thiobencarb, molinate, or pendimethalin (field tests). Combinations of these or other chemicals with propanil may provide additive or synergistic action useful to control R-BYG without increasing rice injury. Such mixtures might also prevent or delay the development of propanil resistance in this weed species.

Keywords: aryl acylamidase; chlorophyll fluorescence; *Echinochloa crus-galli*; insecticide; photosystem II inhibition; synergism

1 INTRODUCTION
The amide herbicide propanil [3′,4′-dichloropropionanilide], a photosystem II (PSII) inhibitor, is the principal herbicide used to control broadleaf and grass weeds, including barnyardgrass (*Echinochloa crus-galli*

* Correspondence to: Robert E Hoagland, USDA-ARS, Southern Weed Science Research Unit, Stoneville, MS 38776, USA
E-mail: rhoaglan@ag.gov
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(L) Beauv), in rice (*Oryza satival* L). It has been widely used in rice production in the southern USA since its introduction in 1962.[1] Estimates indicate that since 1988, 98% of the rice acreage in Arkansas has been treated with one or more propanil applications each year.[2] In 1989, rice producers in Poinsett County, Arkansas, began experiencing barnyardgrass control failure with standard propanil application rates (4.5 kg AI ha^{-1}). Barnyardgrass seeds were collected from these problem fields, and greenhouse tests showed that plants from these seeds were resistant to propanil at doses as high as 200 mM (equivalent to *C* 11 kg ha^{-1}), thus confirming resistance.[1,3] A verification and distribution study conducted in Arkansas in 1991 and 1992 confirmed the presence of propanil-resistant barnyardgrass (R-BYG) in 115 (16 counties) of 138 seed sources.[3] Distribution of R-BYG in rice-growing areas of Mississippi, Texas, and Louisiana has also been demonstrated.[3] More recent studies utilizing chlorophyll fluorescence for rapid detection of R-BYG indicate that its distribution is increasing; ie, it is now in 18 of the 38 Arkansas rice-producing counties.[4] R-BYG and/or propanil-resistant junglerice (*Echinochloa colona* (L) Link) also occur in Columbia, Greece, Japan, and Costa Rica.[5–7]

Propanil is metabolized (deactivated) in rice by the enzyme aryl acylamidase (EC 3.1.1.a) to form 3,4-dichloroaniline (DCA) and propionic acid.[8–11] This pathway is the basis of the selectivity mechanism for barnyardgrass control in rice, since the extremely low levels of aryl acylamidase present in sensitive barnyardgrass (S-BYG) (1/60th of that in rice leaves) are insufficient to detoxify propanil.[10] Aryl acylamidase occurs in various plants, including red rice (*Oryza glaberimma* Steud), a conspecific weed of commercial rice.[12,13] A direct correlation between high aryl acylamidase activity and tolerance to propanil was found in several wild rice (*Oryza*) species.[14] Propanil metabolism is also the selectivity mechanism for green foxtail (*Setaria viridis* (L) Beauv) control in wheat (*Triticum aestivum* L).[15] Elevated levels of aryl acylamidase were found in propanil-resistant junglerice.[16]

Weed resistance to herbicides has developed via several mechanisms. Resistance is generally due to a change in herbicide absorption or translocation, a molecular modification of the herbicide's site of action, or increased metabolic degradation of the herbicide.[17] Our laboratories showed that the resistance mechanism in R-BYG was not differential absorption and translocation of propanil[18] or molecular modification of the propanil site of action,[18] but was due to elevated aryl acylamidase activity.[2]

Aryl acylamidase activity is inhibited by carbamate and organophosphate insecticides such as carbaryl, parathion and methiocarb.[9,10] These insecticides are competitive inhibitors of rice aryl acylamidase[10] and can increase injury when applied in close temporal proximity to, or simultaneously with, propanil.[19] PPG-124 (*p*-chlorophenyl *N*-methylcarbamate) lacks herbicidal activity, but is a propanil synergist via its inhibition of aryl acylamidase.[20]

Aryl acylamidase inhibitors and herbicides that cause effects other than photosynthetic inhibition may be useful in the management of R-BYG when combined with propanil. Thus, we are presently examining the interaction of compounds with propanil to find synergistic or additive actions that can increase R-BYG control without increasing rice injury. To achieve this, field tests (evaluation of herbicidal injury) and laboratory tests (quantification of chlorophyll in excised leaf tissues; measurement of chlorophyll fluorescence to determine PSII inhibition) are being conducted on R-BYG and rice exposed to various rates of propanil + additive.

2 EXPERIMENTAL AND RESULTS

2.1 Field tests

The herbicides anilophos, molinate, pendimethalin, piperophos, quinclorac, thiobencarb and the insecticide carbaryl were applied alone or in combination with propanil to rice and R-BYG at the two-leaf stage. Visual evaluations of herbicide injury were recorded seven to 49 days after treatment.

Among the important synergistic interactions found on R-BYG were propanil + anilophos, propanil + piperophos, and propanil + carbaryl. Carbaryl had not been field-tested as a propanil synergist to control R-BYG, but high concentrations of carbaryl and anilophos combined with propanil caused some rice injury. Pendimethalin also exhibited synergistic interactions. Other important synergistic and/or additive interactions were found at certain application rates when propanil was combined with molinate, quinclorac, and thiobencarb.

2.2 Chlorophyll fluorescence and chlorophyll quantification

Leaf discs were floated on each chemical solution or combination, and PSII photosynthetic inhibition was measured by chlorophyll fluorescence.[4,21] Combinations of propanil + anilophos, carbaryl, methiocarb, piperophos, or PPG-124 provided a high level of PSII inhibition in R-BYG. Fluorescence tests with propanil + methiocarb and PPG-124 (Fig 1) and carbaryl support previous findings that these compounds inhibit aryl acylamidase activity.[10,20] There was little or no effect from combinations of propanil + molinate, pendimethalin, quinclorac, or thiobencarb. Some of these compounds have been shown to have positive interactions with propanil in laboratory and field tests for propanil-resistant junglerice control, ie, propanil + piperophos[22] and propanil + anilophos[23] can control propanil-resistant junglerice without adverse effects to rice.

Chlorophyll in leaf discs of R-BYG exposed to solutions of propanil (200 μM) or propanil + additive (100 μM) under continuous light (300 μE m^{-2} s^{-1}, PAR; 48–60 h) was measured spectrophotometrically

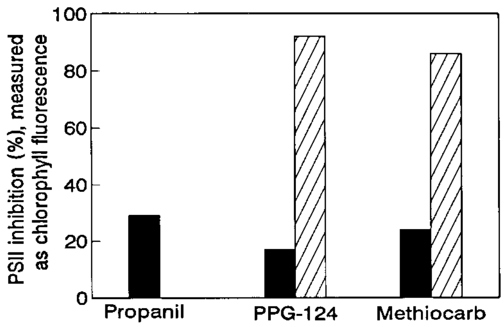

Figure 1. Effect of propanil alone (100 μM) or in combination with PPG-124 (50 μM) or methiocarb (50 μM) on photosynthesis in R-BYG leaf discs, measured by chlorophyll fluorescence. ■ compounds alone; ▨ compound + propanil.

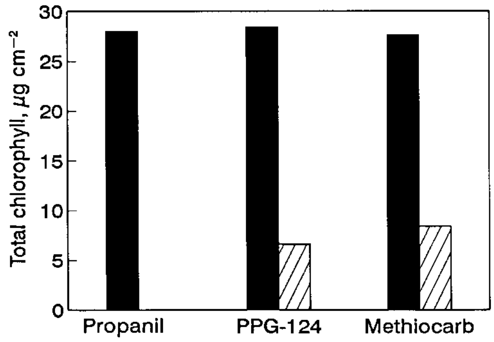

Figure 2. Effect of propanil alone (200 μM) or in combination with PPG-124 (100 μM) or methiocarb (100 μM) on total chlorophyll content of R-BYG leaf discs. ■ compounds alone; ▨ compound + propanil.

after extraction with dimethylsulfoxide. Methiocarb and PPG-124 (tested only in the laboratory) greatly reduced total chlorophyll in R-BYG leaf discs when combined with propanil (Fig 2). In most cases, chemical combinations that reduced chlorophyll also caused substantial R-BYG control (field tests) and high PSII inhibition (chlorophyll fluorescence tests).

Using compounds with different modes of action to control a particular species can reduce the probability of resistance development. Rotation of rice with another crop, such as soybeans, can delay and/or prevent the occurrence of R-BYG. Combinations of some chemicals with propanil can provide additive or synergistic action to increase control of R-BYG without increasing injury to rice. The interactions of other compounds with propanil are being pursued.

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